PhD theses

THE ENCHYTRAEID (OLIGOCHAETA: ENCHYTRAEIDAE) FAUNA OF HUNGARIAN BOTANICAL GARDENS, REGARDING EXOTIC AND INVASIVE SPECIES

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INTRODUCTION

The family of Enchytraeids or potworms is classified in the phylum Annelida, the class Clitellata, the subclass Oligochaeta and the order Enchytraeida. It can be found in very diverse terrestrial and aquatic habitats, so probably this is the most numerous and widespread taxon among the families in the class Clitellata. Species of the family can be found from tropical areas beyond the Arctic circle, in different soils, leaf litter, decayed plat matter, sandy coastline habitats, in organogenic sediment of deep sea or on surface of glaciers. For these reasons, it is not surprising that they can be found in artificial, man-made environments. Such places are greenhouses: enchytraeids arrive at these new habitats with the soil and on the roots of imported exotic plants. The permanent warm temperature and the continuous, copious watering ensure appropriate living conditions for (sub)tropical enchytraeids all year round. They not only survive but also create stable populations.

Since used greenhouse soils are often transferred outdoors, some tolerant species can survive and appear in the indigenous enchytraeid fauna. Transferability of some Oligochaete species with soil into new habitats has been known for about a hundred years. Several scientists investigated this phenomenon in the case of Lumbricids and refer to their eventuality of invasion. Invasive earthworm species are able to modify micro- and mesofauna of the soil: they affect the density and diversity of microarthropods, and the density of enchytraeids.

Despite the fact that the introduction of enchytraeids into new habitats is a resembling and parallel phenomenon to the introduction of earthworms, there were no such investigations on enchytraeids. Filling this gap would be significant for several reasons. On the one hand, it is presumable that changes in the composition of the enchytraeid fauna affect the soil micro- and mesofauna. On the other hand, also because of higher densities of enchytraeids in some habitats one cannot ignore their indispensable and important role in the circulation of soil organic matter.

For these reasons a complex investigation seemed obvious on the enchytraeid fauna of Hungarian greenhouses, which observes potential adventive species, describes new species, and collects knowledge about surviving possibilities of newcomer species.

OBJECTIVES

The main object of my doctoral work was to investigate the presence of adventive enchytraeid species in Hungarian botanical gardens. The principal tasks were, accordingly,

1) to establish which sort of enchytraeid species are living in these habitats,
2) to describe new species to fauna/science, elucidate the taxonomic status of some uncertain species,
3) to execute phylogenetic comparison of the found species.

In order to ascertain which species are susceptible to become invasive, it was necessary

4) to appraise the density of the characteristic greenhouse enchytraeid species,
5) to investigate the reproduction of certain species,
6) to verify the hypothesis that some adventive species can colonize in outdoor conditions.

Besides all these,

7) I attempted to find out mobility patterns of newcomers,
8) I was looking for relationship between the found enchytraeid species and some properties of their habitat soil.

MATERIALS AND METHODS

Faunistics, taxonomy

In course of the research 195 soil samples were collected in 13 Hungarian green houses. Qualitative samplings were carried out with a hand shovel. Quantitative samples for estimating density values were made by a special iron cylinder. Worms were extracted from the soil by O'Connor's wet funnel method and filtered on a 50 μm mesh plankton net. Enchytraeids and leftover soil were put into Petri-dishes and kept refrigerated.

Worms were investigated and identified alive with light microscope. Found species were noted and drawn, digital photos were taken. Since several characteristics can be examined stained only, borax carmine preparations were mounted in euparal.

Molecular methods, phylogenetics

For molecular methods nucleic acids were isolated, thereafter the ITS region of the nuclear rDNA was increased by PCR. Sequences were established and phylogenetic analyses were carried out with Maximum Parsimony, Maximum Likelihood and Bayesian analysis.

Density assessment

Quantitavive samples were taken from green houses of Budapest Zoo, Botanical garden of ELTE University and the Institute of Ecology and Botany, Vácrátót, in a 50 cm diameter of the stock of similar plants species (*Phoenix* spp. (Arecaceae), *Pandanus* spp. (Pandanaceae), *Dieffenbachia* spp. (Araceae), *Musa acuminata* (Musaceae); *Ficus benjamina* (Moraceae)).

Worms were extracted by the aforementioned method. The relation of densities and plant species were established with non-metric multidimensional scaling and canonic correspondence analysis.

Investigation of asexual reproduction of the fragmenting Marionina species

Breeding cultures were maintained on agar-agar and sterilized soil on 3 different temperatures (5 °C, 14 °C, 22 °C). Worms were fed on dried nettle leafs and rolled oats. As an experiment, rye and wheat flour, wheat germ, crushed sunflower seeds were also given. In order to stimulate sexual organs, a special culture-medium were made with genital hormones: gonads of sexually matured specimens of *Henlea nasuta* were removed and a suspension was made with 1% agar-agar. The numbers of fragments were followed in Christensen's breeding cultures.

In vitro extrusion experiments

In 4 series of experiments the relative reproduction of *Enchytraeus bigeminus*, *Henlea ventriculosa* and *Fridericia bulboides* were compared. Species were examined in pairs and together to follow the numbers of offsprings.

Outdoor surviving experiment

The surviving experiments of green house adventive species was carried out in the Botanical garden of ELTE University in a 0,25 m^2 (50×50 cm) enclosed area without any disturbance. Before the study, a qualitative sampling determined the naturally present enchytraeid species. In October 2010, specimens of 3 green house potworms, *Enchytraeus bigeminus, Marionina scintillans* and the fragmenting Marionina species were settled outdoors. 5 samplings were executed in 2011 and 1 sampling in 2012. Each sample's volume was 200 cm^3.

Pedological analysis

Soil moisture, pH and organic matter content (oxidized by vitriolic potassium-dichromate) of the soil samples were measured.

RESULTS

1) The PhD thesis is the first-ever description of the enchytraeid fauna of some Hungarian botanical gardens and green houses. 58 enchytraeid species from 12 genera were found and among these 1 genus and 9 species were formerly not known in the natural Hungarian fauna. 5 species new to science were also found. Some of the identified species have tropical or Mediterranean origin, consequently the importation of adventives seems proved.

2) As a co-author I described the new species *Marionina scintillans* Boros & Dózsa-Farkas, 2008 which was found both in the green house of Budapest Zoo, the Botanical garden of ELTE University and the green house of Institute of Ecology and Botany, Vácrátót.

3) In a comprehensive and detailed study, the invalidity of *Mesenchytraeus "kuehnelti"* Dózsa-Farkas, 1991 was established and the species was synonymised with *Mesenchytraeus pelicensis* Issel, 1905.

4) 4 different morphotypes of *Fridericia aurita* Issel, 1905 were separated based on morphological and molecular biological results of this and former studies.

5) Genetical heterogeneity was established between the populations of *Fridericia maculatiformis* Dózsa-Farkas, 1972 with 4 and 5 preclitellar nephridia.

6) Phylogenetical analysis of the nuclear rDNA ITS region proved that species found in green houses are identical with their counterparts from natural habitats.

7) Densities of enchytraeids were estimated in 3 green houses. It was shown that *Marionina scintillans* has extreme high density compared to other species in their natural habitats. In the case of *Enchytraeus bigeminus* relatively high numbers of individuals were observed.

8) During the faunistic research it was found that a *Marionina* species is able to reproduce asexually by architomy (fragmentation). This ability is unique in the genus and is rare in the family. Reproduction of this species was investigated: data about size, number and development intensity of fragments were collected.

9) The effect of adventive enchyraeids on native potworms was also investigated. This experiment proved that *Enchytraeus bigeminus* reproduce more intensively in breeding cultures than other enchytraeids and decreases the number of descendants of remaining species consequently.

10) Another experiment verified that *Enchytraeus bigeminus* and *Marionina scintillans* are able to survive dry summer seasons and winter frosts outdoors if resettled from a green house. Considering the above mentioned features of *E. bigeminus*, this species can become invasive.

During the work attempts were made to follow the mobility patterns of newcoming species. These investigations were unsuccessful because of the imperfect documentation of botanical gardens and barter between them.

11) I proved with exhaustive sampling that adventive enchytraeids are not spread with used humus or purchased potting compost.

12) The results of pedological analysis were not relation to the find species.

CONCLUSIONS

As a result of my doctoral work the enchytraeid fauna of some Hungarian botanical gardens became known. It is considered to be proved that adventive enchytraeids are able to get into the green houses of temperate climate. On the basis of these it would be deserve to extend the investigations to other botanical gardens.

Taxonomical experiences showed that simultaneous use of morphological and phylogenetical methods is the most effective. However, using of different sequences is necessary, for instance the results of ITS region in itself is not sufficient.

Further investigations of the reasons and effects of extreme high densities established in artificial environment can result new consequences in the ecology of soil micro- and mesofauna.

Breeding cultures of fragmenting *Marionina* species is maintained to observe sexual organs and sexual reproduction in the future.

Results of in vitro experiments refer to further study of fragmenting enchytraeids to ascertain the successfulness of this way of multiplying compared to sexual reproduction.

Outdoor surviving experiments showed that some adventive enchytraeids can survive and certain species can gain ground outdoors. Accordingly, the appearance of newcomer potworms is expected in the Hungarian fauna, for instance *Enchytraeus bigeminus* can invade native habitats.

PUBLICATIONS

Publications in the subject of the doctoral thesis:

BOROS, G. (2012): First record of reproduction by fragmentation in the genus *Marionina* (Oligochaeta: Enchytraeidae). Zoology in the Middle East (in press).

IF: 0,412

CECH, G., **BOROS, G.**, DÓZSA-FARKAS, K. (2011) Revision of *Bryodrilus glandulosus* (Dózsa-Farkas, 1990) and *Mesenchytraeus kuehnelti* Dózsa-Farkas, 1991 (Oligochaeta: Enchytraeidae) using morphological and molecular data. Zoologischer Anzeiger doi:10.1016/j.jcz.2011.09.005

IF: 1,846

BOROS, G. (2011**):** Outdoor surviving experiment of three green house enchytraeid species (Oligochaeta: Enchytraeidae). Opuscula Zoologica Budapest 42 (2): 207-210.

BOROS, G. (2010) Enchytraeids from potting compost purchasable in Hungarian retail trade. Opuscula Zoologica Budapest 41 (2): 237-240.

Boros, G. & Dózsa-Farkas, K. (2008) *Marionina scintillans* sp. n., a new enchytraeid species (Annelida: Oligochaeta) from Hungarian green houses. Acta Zoologica Academiae Scientiarum Hungaricae, 54 (2): 113-123.

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Other publications

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IF: 0,412

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Dózsa-Farkas K. & **Boros, G.** (2005): *Achaeta antefolliculata* sp. n., a new enchytraeid species (Oligochaeta: Enchytraeidae) from the rock grassland of the Sas-hegy in Hungary. Acta Zoologica Academiae Scientiarum Hungaricae 51 (4): 279-285.